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A review on electrochemical methods for trace metal speciation in environmental media

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Abstract

Trace metal speciation is key to understand/predict bioavailability and potential toxicity of metals to biota and will, undoubtedly, be incorporated in future regulations. Electroanalytical methods have a role to play in such development: they offer a wide range of advantages such as speed, portability, economy, solid interpretation backgrounds and low limits of quantification. This review focusses on three selected stripping techniques: Competitive-Ligand Exchange - Cathodic Stripping Voltammetry (CLE-CSV), Scanned Stripping Chrono-Potentiometry (SSCP) and AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) reporting their working principles, characteristics (strong and weak points) and recent applications to systems of environmental relevance (such as seawaters, freshwaters or soil extracts).

Keywords: speciation, availability, electroanalysis, electrochemistry, heavy metal, CLE-CSV, SSCP, AGNES

28 **List of nomenclature and abbreviations:**

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AdCSV	Adsorptive Cathodic Stripping Voltammetry
AFS	Atomic Fluorescence Spectroscopy
AGNES	Absence of Gradients and Nernstian Equilibrium Stripping
APDC	Ammonium 1-pyrrolidinedithiocarbamate
ASV	Anodic Stripping Voltammetry
BiFE	Bismuth Film Electrode
BLM	Biotic Ligand Model
CLE	Competitive Ligand Exchange
CSV	Cathodic Stripping Voltammetry
DHN	2,3-dihydroxynaphthalene
DMEM	Dulbecco's modified Eagle's medium
DMG	Dimethylglyoxime
DMT	Donnan Membrane Technique
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DPP	Differential Pulse Polarogram
EDTA	Ethylenediaminetetraacetic acid
EPPS	4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid
EPS	Extracellular polymeric substances
EQS	Environmental Quality Standard
FIAM	Free Ion Activity Model
GSH	Glutathione
HEPES	N-2-Hydroxyethylpiperazine-N'-2'-ethanesulfonic acid
HMDE	Hanging Mercury Drop Electrode
HS	Humic Substances
<i>I</i>	Ionic strength
ISE	Ion Selective Electrode
LOD	Limit of Detection
MES	2-(N-morpholino)-ethanesulfonic acid
MOPS	3-(N-morpholino)-propanesulfonic acid
NICA	Non-Ideal Competitive Adsorption
NN	1-Nitroso-2-naphtol
NOM	Natural Organic Matter
NP	Nanoparticles
NTA	Nitrilotriacetic Acid
OECD	Organisation for Economic Cooperation and Development
PDCA	Pyridinedicarboxylic acid
PNIPAM	Poly(N-isopropylacrylamide)
POPSO	Piperazine-1,4-bis(2-hydroxypropanesulfonic acid)

QD	Quantum Dot
RT	Resin Titration technique
S	Salinity
SA	Salycilaldoxime
SCP	Stripping ChronoPotentiometry
SIA	Sequential Injection Analysis
SPE	Screen Printed Electrode
SSCP	Scanned Stripping ChronoPotentiometry
SSV	Scanned Stripping Voltammetry
TAC	2-(2-Thiazolylazo)-p-cresol
TMFE	Thin Mercury Film Electrode
UV	Ultra Violet
VGME	Vibrating Gold Micro Electrode
VM	Visual Minteq

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Introduction

Speciation (i.e. the distribution of an element among its different chemical forms[1]) has been recognised as a key factor for trace metal (bio)availability to biota, as postulated by hegemonic ecotoxicological paradigms such as the Free Ion Activity Model (FIAM) [2] or the Biotic Ligand Model (BLM)[3], both assuming that the free ion is the only bioavailable species. The limits of these models have recently been reviewed [4], highlighting numerous cases where specific fractions of metal complexes can be internalized, either directly or through the dissociation of labile complexes. Thus, there is a need to design, develop, apply and interpret analytical techniques able to provide reliable speciation information [5-7] that can be used by regulatory bodies. Although there are numerous non-electrochemical techniques [5-13], those that are provide attractive possibilities such as speed of analysis, low-cost and miniaturization in view to on-site (and in-situ) measurements [14], and can also provide thermodynamic (i.e. equilibrium) information (such as the amount of the free metal, the amount of complexing sites, the stoichiometry of complexes and their stability constants [15]) as well as dynamic information (such as kinetic rate constants and diffusion coefficients [16]). However, the correct signal interpretation can be difficult in natural media [16;17]. Stripping techniques, due to their low detection limits, are well suited for environmental analysis. They are two-stage techniques, where the first stage is a deposition step that accumulates the analyte (at the electrode surface or in its volume), followed by a second stage that quantifies this analyte. Anodic Stripping Voltammetry (ASV), in its multiple variants, is the most popular and has the advantage to possibly be measuring a potentially bioavailable fraction of the metal, as shown again recently [18;19].

This critical evaluation focusses on three techniques that are known powerful tools for metal speciation: AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and SSCP (Scanned Stripping ChronoPotentiometry), that are relatively recent techniques, together with the more established CLE-CSV, Competitive Ligand Exchange (or Equilibration) - Cathodic Stripping Voltammetry. Principles, recent applications, weak and strong points are reviewed.

CLE-CSV (Competitive-Ligand Exchange - Cathodic Stripping Voltammetry)

CLE-CSV is an equilibrium-based method that determines complexation parameters, namely ligand concentrations and conditional stability constants, allowing indirect determination of the free metal ion concentration. The method consists in the addition of a ligand (L_{add}) that forms an electroactive complex ML_{add} with the metal M of interest. Once equilibrium between the natural ligands (L_x) and L_{add} is achieved (typically few hours), quantification of ML_{add} adsorbed on the sensor (Figure 1) by CSV in a minimum of 12 aliquots at increasing concentrations of M leads to a titration curve ($I_p(ML_{add})$ vs $c_{T,M}$) that can be fitted with the presence of up to typically 2 classes of ligands of specific concentrations $[L_x]$ and conditional stability constant K_x' . Recent CLE-CSV studies published in the last 2 years were applied almost exclusively to marine systems, looking at the speciation of biologically important metals, mostly Cu[20-29] and Fe[30-36], but also Zn[37;38] or Co [39]. The analytical method is powerful and can give insights into potential biological limitation (e.g. free Cu^{2+} limitation for Cu-dependent ammonia oxidising archaea [40]), toxicity (e.g. high free Cu^{2+} in coastal waters[41]) or for studying the effect of ocean acidification on future Cu and Fe speciation [27]. It is also used to indirectly look at the biogeochemical cycling of the ligands, from their origins (e.g. sediments [22], peat [21], biological productivity in

water column [29], stormwater[42]), and identity (e.g. humics [24], polysaccharides[43]) to their seasonal [31] and spatial [35;36] variations, as well as stability [21].

Recent analytical developments to note include the catalytic detection of Fe by oxygen in presence of SA [44] and the use of reverse titrations [45;46], where the concentration of the ligand, not that of the metal, is varied. New methods for the determination of humic substances, HS (based on the adsorption and quantification of Cu-HS complex at the Hg drop electrode [28], similarly to the Fe-HS method[47]), have demonstrated that the same HS are involved in the complexation of both Cu and Fe in coastal waters of Liverpool Bay, accounting for 97 and 65% of the total ligands for Fe and Cu, respectively [28]. This competition for ligands implies that the complexation of Fe-HS might be dependent on Cu levels, or other metals, and vice versa. Various fitting methods can be used to determine speciation parameters from the titration curves (current vs. total metal concentrations). However, both the experimental [48] and fitting [49;50] procedures are far from trivial and latest recommendations [49] are, if time allows, to run multiple titrations at different detection windows (i.e. with different concentrations of the added ligand e.g. [51;52]) to decrease errors. Freely available software such as ProMCC [53] or others (see [49] and references therein) are available to help fitting the data correctly. However, no matter how well data are fitted, CLE-CSV does not give any indication related to potential kinetics and bioavailability of the various complexes. When internalization is fast enough to develop a free metal concentration profile close to the consuming interface, fast dissociating organic complexes (e.g. Cu-HS substances [54]) may also participate to the metal flux to organisms and methods based on anodic stripping voltammetry can estimate this bioavailable fraction [19;55].

CSV in presence of relatively high concentration of added ligand(s) is also widely used as a detection technique and is very helpful in gaining insights into the cycling of various metals, such as that of Zr, Mo, V and Ti at increasing salinities [56]. Although the Hg drop electrode is still used for developments of new CSV methods (e.g. for Ga [57], Te[58] or Fe [59]), a large amount of research is now devoted to greener and more user-friendly solid and/or film electrodes. Latest development for analysis of water samples include methods for Ni [60], Ag[61], Co [62], Mn[63], V[64;65], U[66], Ga[67] and Cr[68]. These methods are developed using common carbon paste electrodes [60;61] or screen-printed ones [69;70], but also use more recent substrates such as e.g. lithographically produced Bi microelectrode array [71], tin-bismuth alloy[62;66] or indium-oxide [63]. Although progress is coming fast, up to now, only the Hg drop electrode is used for CLE-CSV speciation studies (due to yet unrivalled stability over the time required to perform a titration).

SCP/SSCP (Scanned Stripping ChronoPotentiometry)

Stripping Chronopotentiometry (SCP) was developed in the early 2000's by Town and van Leeuwen [72] mainly to address the problems caused by electrodic adsorption of organic matter in trace metal analysis in environmental waters. The main difference in this technique is that, in the stripping step, a sufficiently small reoxidation current is used such that a small diffusion gradient inside the electrode is created. Therefore full depletion of the accumulated analyte is accomplished effectively, and the integrated recorded signal is essentially proportional to the accumulated charge, almost immune from electrodic adsorption of organic matter, distorted peaks [73] or heterogeneity of metal complexes [74]. SCP truly reaches its potential when a thin mercury film electrode is used [75] due to the low detection limit associated with these electrodes,

and ever since full depletion is always maintained, allowing the decrease of both the analysis time and the oxygen interference [76].

One of the limitations of SCP is the absence of an expression relating the peak potential and the metal complex speciation in the matrix solution. This led to the development in mid-2000's of an extension named Scanned Stripping Chronopotentiometry (SSCP), which is composed from a series of individual measurements performed at different deposition potentials (E_d), varying from values where no metal reduction occurs to the diffusion limited region where the free metal concentration at the electrode surface is nil [77].

In SSCP, the shift in half-wave deposition potential in the presence of complexing agents species can be interpreted straightforwardly, analogous to the DeFord-Hume expression for a voltammetric wave [78], while the decrease in limiting transition time (i.e. the stripping time when accumulation is under diffusion limited conditions) provides information on the dynamic nature of the metal complexes, namely their diffusion coefficients and association rate constants [79]. Additionally, the slope variation of the SSCP wave provides information on the degree of heterogeneity of the complexing ligands [80].

SCP in environmental analysis was reviewed in 2007 [81], while SSCP applications in the development of chemodynamics theory and applications in seawater analysis were reviewed in 2012 [16]. We are focusing here on published works from 2012 which can be clustered in four groups: SCP/SSCP technical and methodological developments; trace metal interaction with humic matter; nanoparticle studies and, finally, trace metal bioavailability studies (see Table 1 for applications).

SSCP was first used with solid electrodes for the study of Pb(II) speciation using bismuth electrodes [82]. Pb(II) speciation in seawater required attention to the correct

interpretation of the calibration, due to the formation of PbCl_x complexes [83], while ligand size polydispersity was investigated using the binding of Pb(II) and Cd(II) to three different sizes of otherwise identical polymers [84]. The study of Cd(II) binding by clay minerals provided interesting dynamic information [85] and, finally, the effect of temperature in the lability of Cd(II) and Pb(II) complexes was investigated [86].

SCP methods were developed for the Hg(II) analysis using Sequential Injection Analysis (SIA) and screen printed gold electrodes [87]; for the metal detection in the acceptor solution of a DMT device [88]; for sulphide determination in hydrothermal seawater samples using a vibrating gold micro-wire [89]; and for the speciation of Cd(II) and Pb(II) in the acidified ultrafiltrated solution ($<1\text{kDa}$) [90].

SSCP was used in several works investigating the binding of trace metals with humic matter, namely the binding of Cu^{2+} and Ni^{2+} with aquatic fulvic and humic acids [91], the effect of Ca^{2+} on the chemodynamics of Pb(II) complexation by humic acid (HA)[92] or the effect of electric condensation on the HA binding of Cd^{2+} , Pb^{2+} , Cu^{2+} [93], among others.

There are several examples of SCP and SSCP studies to elucidate the speciation of metal ions in presence of nanoparticles and/or to study the dissolution of the nanoparticles. For instance, SSCP was used to investigate the structural effects on Cd(II) binding to multi-responsive carboxylated PNIPAM particles (temperature, pH, ionic strength) [94], the role of charged polymer coatings of nanoparticles on the trace metal speciation [95], the impact of electrostatics on the binding of Pb(II) and Cd(II) with highly charged nanoparticles [96] and the dissolution of Cd-based quantum dots in seawater [97].

There are several examples of SSCP utilization to determine trace metal speciation in exposure solutions of biouptake experiments, like the study of Cd(II) availability to

183 *Saccharomyces cerevisiae* in presence and absence of polyhydroxyfullerenes [98],
 184 while SCP was used, for example, to follow the bulk Cd(II) depletion due to biouptake
 185 by *Pseudomonas putida* [99]).

186 **AGNES (Absence of Gradients and Nernstian Equilibrium** 187 **Stripping)**

188 AGNES technique provides direct access to the free metal ion concentration in solution
 189 via a two-stage voltammetric procedure [100]. In the first stage, the metal ion M^{n+} in
 190 solution is reduced and amalgamated in the mercury electrode M^0 due to the application
 191 of a potential program [101] always ending with the deposition potential E_1 (for a
 192 sufficiently long time). By the end of the first stage, equilibrium has to be reached: i)
 193 absence of gradients in the concentration profiles of the reduced and oxidised species on
 194 both sides of the electrode interface and ii) metal concentrations at both sides of the
 195 electrode surface are related through Nernst relationship

$$196 \quad Y = \frac{[M^0]}{[M^{n+}]} = \exp\left[-\frac{nF}{RT}(E_1 - E^{o'})\right] \quad (1)$$

197 where Y is the gain (or preconcentration factor), F the Faraday constant, R the gas
 198 constant, n the number of electrons, T the temperature and $E^{o'}$ the standard formal
 199 potential of the redox couple. If at equilibrium, the amount of reduced species is directly
 200 proportional to the free metal ion concentration in the solution. The aim of the second
 201 stage is, thus, to quantify that amount of reduced M^0 accumulated. In the variant
 202 AGNES-I, M^0 is reoxidised under diffusion-limited conditions and the current (I) is
 203 measured. Other variants for the second stage quantify the stripped charge (Q) in the
 204 reoxidation step at a fixed potential (AGNES-Q) [102] or along a linear reoxidation
 205 potential scan (AGNES-LSV) [103]. A fourth variant (AGNES-SCP), especially useful
 206 when electroactive metals other than the analyte are present, computes the charge from
 207 the recorded potentials needed to sustain a fixed stripping current [104]. The state of

equilibrium at the end of the first stage can be checked by constant responses with longer deposition times at E_1 .

One of the most attractive characteristics of AGNES is its simplicity: there is a linear relationship between the measured signal and the free metal concentration. In AGNES-I

$$I = Y\eta[M^{n+}] \quad (2)$$

while in the other variants

$$Q = Y\eta_Q[M^{n+}] \quad (3)$$

where η and η_Q are proportionality factors found in calibrations (except in the case of indium [105]).

Due to the equilibrium situation, the free concentration determination is not affected by complexation, precipitation or induced electroodic adsorption [103]. A local free metal concentration in non-purged solutions can be found [102].

AGNES has been widely applied with the hanging mercury drop electrode (HMDE).

Other mercury-based electrodes used are the Hg-Ir microelectrode [106], the rotating disk electrode [107] and the screen printed electrode, SPE [108]. The SPE is specially indicated for *in situ* applications [109]. Mercury-free options are the bismuth film deposited on the surface of a glassy carbon solid electrode [110] or the Vibrating Gold Microwire electrode (VGME) [111].

The capability of AGNES to determine free metal ion concentration has been validated with other techniques, such as ISE [103], DMT [8] [112], SSCP [113], CLE-CSV [38] as well as with speciation codes [101;114].

A list of applications is given in Table 1. Very low ionic strength systems, like river waters, can be dealt accurately with AGNES due to the low product of residual current times resistance when equilibrium is reached[90;102;115]. Higher ionic strength systems, such as estuarine [38] and seawater samples [83;116], have also been analysed

with AGNES. The extent of complexation of metals to Dissolved Organic Matter (DOM) [117-120], clays [85] or nanomaterials -from latex [96;113] to TiO₂ nanoparticles [121;122] - has been assessed. The solubility of different nanomaterials has been evaluated, i.e. CdTe/CdS quantum dots [123] and ZnO nanoparticles [124;125]. AGNES has also contributed to the elucidation of toxicity mechanisms, i.e. ZnO nanoparticles to *Daphnia magna* [126].

Conclusions and perspectives

The three selected techniques have been successfully applied to a large number of environmental systems. Table 1 presents an exhaustive list of AGNES applications with selected recent applications of SSCP and CLE-CSV. On the other hand, table 2 outlines *caveats* and drawbacks of each of those techniques, so that a comparison between them is possible. As general conclusions of such comparison, one can say: i) CLE-CSV is the most established technique, currently used mostly for Cu and Fe speciation in marine systems but has also been used for few other elements (e.g. Zn, Ni, Co); on the other hand, the adsorptive CSV method is applicable for the detection of numerous elements and CLE-CSV is, thus, also potentially applicable to those, assuming that the complexation between added ligand and metal of interest is well known. In CLE-CSV, sample throughput is limited by the often-long experimental time; the use of specifically developed fully automated system for the detection (and possibly the titration) would certainly help in this respect; ii) AGNES is powerful in measuring directly the free ion concentration; once limited to typical amalgamating elements (Cd, Pb, Zn), there are now promising results for less typical ones (Sn, In, Sb, Cu) as well as future candidates (e.g. Tl, Ga) and great potential development using solid electrodes (ex: Cu on gold and Pb on BiFe); iii) SSCP and CLE-CSV provide indirect access to the free ion concentration (as well as stability constant and ligand concentration), but the modelling

is based on several assumptions; iv) SSCP provides dynamic information on complexes. v) To avoid changes in the original speciation due to e.g. purging, mixtures of N₂ and CO₂ (or other strategies) might be used. vi) Miniaturization of equipment in the future should reduce the current minimum amount of needed sample (of the order of 10 mL). vii) The study of the impact of temperature effects on the application of the techniques have just started. viii) The improvement of mercury film electrodes (especially when combined with more effective and reproducible decreases of the diffusion layers due to some kind of stirring or moving) will lower their limit of detection. ix) 10% uncertainty in the retrieved concentration values is usual. x) Even though there is extensive published documentation, training with experienced practitioners is strongly recommended before using any of the 3 techniques. These techniques have been extensively validated (independently and against each other), though further inter-comparison work in specific systems is timely. They can be complementary when applied to the same system (for instance coupling of SSCP and AGNES is important in environmental studies due to the presence of heterogeneous ligands [93;113;127]). Electroanalytical techniques yield physicochemical information on the exposure medium, but this must be correlated with bioavailability and toxicity in interdisciplinary studies; these are needed for future implementation of adapted EQS at local level.

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Tables

Table 1. Compilation of AGNES and recent CLE-CSV and SSCP applications to selected systems, arranged in chronological order and by alphabetical order of the first author. Unless stated otherwise, $T=25^{\circ}\text{C}$

Analyte	System	Technique	Electrode Type	pH	$I/\text{mol L}^{-1}$ (electrolyte)	Range of concentrations	Comments	Reference
Cd	NTA	AGNES	HMDE	4.7	0.1 KNO_3	$[\text{Cd}^{2+}] = 0.14$ to $1.4 \mu\text{mol/L}$	Proposal of the basic principles of AGNES. Introduction of variant AGNES-I and 1 Pulse.	[100]
Zn	NTA, EDTA, Oxalate	AGNES	HMDE	4.0 to 6.2	0.1 KNO_3	$[\text{Zn}^{2+}] = 0.029$ to $23 \mu\text{mol/L}$	AGNES measurements are not hindered by some irreversibility or precipitation. AGNES 2P is introduced.	[101]
Pb	NTA PDCA	AGNES	HMDE	pH 3.0 to 6.0 pH 2.8 to 7.0	0.1 KNO_3	$[\text{Pb}^{2+}] = 0.010$ to $1.0 \mu\text{mol/L}$ $[\text{Pb}^{2+}] = 1.2$ to 88 nmol/L	Values of AGNES crossvalidated with RT	[114]
Cd Zn	Aldrich Humic Acid	AGNES	HMDE	4 to 7	0.1 KNO_3	$[\text{Cd}^{2+}] = 0.017$ to $96 \mu\text{mol/L}$ $[\text{Zn}^{2+}] = 78 \text{ nmol/L}$ to	NICA-Donnan fitting of AGNES results.	[117]

						95 μM		
Zn	Coastal Mediterranean seawater	AGNES	HMDE	8.2	natural ionic strength	$[\text{Zn}^{2+}] = 1$ to 3 nmol/L	Introduction of the shifted blank for AGNES. First application of AGNES to seawater. Use of 2P variant.	[116]
Pb	PDCA	AGNES	mercury-coated iridium microelectrode	6.1	0.1 KNO_3	$[\text{Pb}^{2+}] = 0.0040$ to $1.2 \text{ } \mu\text{mol/L}$	Access to lower free ion concentrations with smaller electrodes.	[106]
Zn	Wine	AGNES	HMDE	3.6	natural ionic strength	$[\text{Zn}^{2+}] = 0.45$ to $0.72 \text{ } \mu\text{mol/L}$	Introduction of the EDTA blank. Methodology for hydroalcoholic systems.	[128]
Pb	Latex nanoparticles	AGNES and SSCP	HMDE	5.1 to 7.1	0.01 NaNO_3	$[\text{Pb}^{2+}] = 0.25$ to $1.0 \text{ } \mu\text{mol/L}$	Values of AGNES crossvalidated with SSCP and ISE.	[113]
Pb	Purified Aldrich Humic Acid	AGNES	HMDE	4 to 9	0.1 KNO_3	$[\text{Pb}^{2+}] = 13 \text{ pmol/L}$ to 0.11 mmol/L	Interpretation of Pb binding through the Conditional Affinity Spectrum distributions.	[118]
Zn	Cu and Oxalate	AGNES	HMDE	6	0.05 KNO_3	$[\text{Zn}^{2+}] = 0.6$ to 2.3 mmol/L	$[\text{Zn}^{2+}]$ measured in presence of Cu. The impact of intermetallic compounds was evaluated.	[129]
Zn Cd	analyte and background electrolyte	AGNES	HMDE	3 to 5	0.05 to 1.0 KNO_3	$[\text{Zn}^{2+}] = 9.4 \text{ } \mu\text{mol/L}$ to 70 mmol/L	Effects of high metal ion concentrations. Response function charge might be more accurate than current (AGNES-I). Introduction of AGNES-Q.	[130]
Cd	NTA/glycine/MOPS NTA/citric	AGNES and SSCP	HMDE	8.00	0.1 NaNO_3	$[\text{Cd}^{2+}] = 2.7$ to 2.9 nmol/L $[\text{Cd}^{2+}] = 2.0$	By unambiguously providing the free metal ion concentration, AGNES contributed to the understanding of the interplay of labilities.	[131]

	acid/MOPS					to 2.9 nmol/L		
Pb	Latex nanospheres IDA	AGNES	TMFE	5.50 to 6.12 5.5	0.01 NaNO ₃	[Pb ²⁺]= 0.087 to 0.28 µmol/L [Pb ²⁺]= 0.029 to 0.18 µmol/L	Use of the Rotating Disc Electrode to reduce the deposition times.	[107]
Zn	Besòs river water	AGNES	HMDE	7.7	natural ionic strength	[Zn ²⁺]= 12.8 nmol/L	First implementation of AGNES to a natural freshwater.	[132]
Cd, Zn, Pb	Oxalate	AGNES	HMDE, SPE	2 to 7	0.1 KNO ₃	[Cd ²⁺]= 0.062 to 1.1 µmol/L [Zn ²⁺]= 6.8 nmol/L to 0.57 µmol/L	Suitability of AGNES-Q for SPE.	[108]
Cd, Zn, Pb	Oxalate NTA	AGNES	HMDE, SPE	4.8 and 6	0.1 KNO ₃	[Cd ²⁺]= 53 to 162 nmol/L	Introduction of the variant AGNES-SCP.	[104]
Pb	Hydrophobic, transphilic and hydrophilic DOM	AGNES	HMDE	4 to 9	0.1 KNO ₃	[Pb ²⁺] = 0.11 nmol/L to 10 µmol/L	Evaluation of the binding properties of each DOM fraction (extracted from effluent of a wastewater treatment plant and from the river Seine) to Pb. General flow diagram to optimise gains and deposition times.	[119]

Zn	Rhine water	AGNES	HMDE	7.9	natural ionic strength	$[\text{Zn}^{2+}] = 84 \text{ nmol/L}$	Values of AGNES crossvalidated with DMT.	[8]
Zn	Dutch soil extracts			4.9 to 6.8	10 mM CaCl_2	$[\text{Zn}^{2+}] = 0.8 \text{ to } 2.4 \text{ } \mu\text{mol/L}$		
Zn	ZnO nanoparticles	AGNES	HMDE	7.5 to 8.8	0.1 KNO_3 or KCl	$[\text{Zn}^{2+}] = 0.83 \text{ } \mu\text{mol/L to } 0.64 \text{ mmol/L}$	The kinetics of dissolution of the nanoparticles could be followed in few-minutes intervals.	[124]
Zn Cd	MOPS, Glycine MES, NTA	AGNES	SPE	7.6 5–6.7	0.01 KNO_3 and MES	$[\text{Zn}^{2+}] = 0.112 \text{ to } 0.759 \text{ } \mu\text{mol/L}$ $[\text{Cd}^{2+}] = 0.45 \text{ to } 1.31 \text{ } \mu\text{mol/L}$	Model for the increase of local pH in the vicinity of the electrode when the solution is not purged. Free local metal concentration can be estimated from AGNES measurements.	[115]
Zn	Glycine	AGNES	HMDE	4–7.5	0.001 to 0.1 KNO_3	$[\text{Zn}^{2+}] = 0.113 \text{ to } 3.07 \text{ } \mu\text{mol/L}$	Low salinity systems can be accurately tackled using DPP peak potentials at higher ionic strengths to compute AGNES gains.	[102]
Zn	Wine	AGNES	HMDE	3.4	0.0560 KNO_3	$[\text{Zn}^{2+}] = 1.49 \text{ } \mu\text{mol/L to } 0.0182 \text{ mol/L}$	Determination of the complexation capacity of wine. Measurements and calibrations at different ionic strengths.	[133]
Cd	Polyacrylate-stabilized CdTe/	AGNES and SSCP	HMDE	4.5 to 8.5	0.01 NaNO_3	$[\text{Cd}^{2+}] = 0.01 \text{ to } 1.0 \text{ } \mu\text{mol/L}$	Evaluation of Cd dissolution from the QD between pH 4.5 to 8.5 and exposed to different concentrations of citric acid, glycine and	[123]

	CdS quantum dots (QD). Effect of citric acid, glycine and histidine.						histidine.	
Cd and Pb	highly charged carboxylated latex nanoparticles	AGNES and SSCP	TMFE	6 to 7 for Cd 5 to 6 for Pb	NaNO ₃ from 6 to 100 mM	[Cd ²⁺]= 1.0 µmol/L [Pb ²⁺]= 1.0 µmol/L	Study of the electrostatic effects on the dynamic behaviour of Pb and Cd binding to highly charged nanoparticles.	[96]
Fe	Celtic Sea for method development	CLE-CSV	HMDE	8.0	S = 35	c _{T,Fe} = 3 nmol/L	5 µmol/L SA + Borate buffer. Catalytically enhanced Fe peak through the use of dissolved oxygen.	[44]
Zn	<i>Daphnia magna</i> in OECD medium	AGNES	HMDE	7.8–8.2	ionic strength of the OECD medium	[Zn ²⁺]= 0.0046 to 2.4 µmol/L	Role of released free Zn concentration in the toxicity of ZnO NP to <i>Daphnia magna</i>	[126]
Pb	Seawater NOM 2 mg/L	SSCP and AGNES	HMDE	7.0 and 8.6	seawater	[Pb ²⁺]= 0.6 µmol/L	Combined study of Pb in seawater by SSCP and AGNES. Influence of lead chloride in the SSCP signal interpretation.	[83]
Cd	Iodide	AGNES	HMDE	6	0.1 KNO ₃	[Cd ²⁺]= 0.82 to 7.8 µmol/L	Free metal concentration correctly determined in presence of electrodic adsorption. General classification of variants.	[103]
Cd	polyacrylic acid			4 to 7	0.1 KNO ₃	[Cd ²⁺]= 0.79 nmol/L		
Pb	xyleneol			6.1	0.1	30 to mmol/L [Pb ²⁺]= 0.46		

Zn	orange ZnO nanoparticles			8.3	KNO ₃ 0.1 KCl	to 2.6 μmol/L [Zn ²⁺]= 10 μmol/L		
Cu	Puget Sound, Washington	CLE- CSV	HMDE	Natural pH (from 7.3 to 8.4)	ca. 22 < S < 30	c _{T, Cu} = 5.5 nmol/L to 12.5 nmol/L	5 μmol/L SA with MOPS, HEPES or EPPS. Show that Cu limitation may be the reason for slow nitrification rates because of limiting Cu- dependent ammonium oxidising archaea.	[40]
Zn	ZnO nanoparticles	AGNES	HMDE	7.7	ionic strength of the DMEM medium	[Zn ²⁺]= 0.053 to 2.5 μmol/L	ZnO NP solubility in the dispersion media, DMEM, under cell culture conditions (37 °C, 5% CO ₂).	[125]
Pb Cd	Clay (Wyoming Montmorillonite SWy-2)	AGNES and SSCP	TMFE	5.5 to 7.1 3.2 to 7.2	0.005 NaNO ₃	[Pb ²⁺]= 0.1 to 1.0 μmol/L [Cd ²⁺]= 0.1 to 1.0 μmol/L	Determination of diffusion coefficient of clay nanoparticles. Determination of binding parameters of Cd and Pb. Assessment of lability and heterogeneity.	[85]
Cu	Seawater Tasman sea	CLE- CSV	HMDE	8.1	34.4 < S < 35.6	c _{T, Cu} = ca. 0.3 to 3.3 nmol/L	0.5 μmol/L SA + 1.9 mmol/L EPPS. Production of ligands attributed to cyanobacteria <i>Synechococcus spp</i> and coccolithophores.	[29]
Cd	TiO ₂ nanoparticles	AGNES and SSCP	TMFE	7.0 and 7.5	0.01 NaNO ₃	[Cd ²⁺]= 0.37 to 0.71 μmol/L	Assessment with AGNES of the impact of TiO ₂ NP addition on [Cd ²⁺] and its toxicity for <i>Corbicula fluminea</i> .	[121]
Cu	Estuarine and Coastal	CLE- CSV	HMDE	8.2	18.8 < S < 32.2	c _{T, Cu} = Cu from 11.6	5 μmol/L SA (Fe)/ 1, 2 or 10 μmol/L SA (Cu) + 10 mmol/L borate buffer.	[28]

Fe					$4.9 < S < 18.8$	to 54.5 nmol/L $c_{T,Fe}$ from 4.9 to 86 nmol/L	HS responsible for almost 100% of Fe complexation, and 70% for Cu. Same HS ligands are involved in the complexation of Cu and Fe.	
Co	Eastern Tropical North Atlantic	CLE-CSV	HMDE	9.1	$34.5 < S < 36.5$	$c_{T,Co}$ up to 80 pmol/L	0.2 or 2 $\mu\text{mol/L}$ Nioxime (or DMG) + 32 $\mu\text{mol/L}$ bromate + NH_3 buffer. Co(II) complexed with weak organic ligands, while Co(III) is complexed to strong inert ligand.	[39]
Fe	North Atlantic Geotraces cruise	CLE-CSV	HMDE	8.2	$34.5 < S < 38$	$c_{T,Fe}$ from 0.3 nmol/L up to typically 1.5 nmol/L	25 $\mu\text{mol/L}$ SA + Ammonium-borate buffer.	[35]
Fe	Estuarine and nearby coastal waters	CLE-CSV	HMDE	8.2	$0 < S < 26$	$c_{T,Fe}$ between 7 and 131.5 nmol/L	SA (9 to 33 $\mu\text{mol/L}$) + borate buffer. Multiple analytical windows. Flocculation of weak ligands at $S < 7$ while strong ligand present at all salinities. [Fe] and [L] tightly coupled along the whole salinity gradient.	[34]
Fe	West Atlantic (North & South) Geotraces cruise	CLE-CSV	HMDE	8.05	$34 < S < 37.4$	$c_{T,Fe}$ from 0.3 nmol/L up to typically 1.8 nmol/L	10 $\mu\text{mol/L}$ TAC + NH_3 /borate buffer. Study shows that Fe ligands have a surprisingly constant $\log K'$ of 22.3 throughout most of the West Atlantic.	[36]
Cu	Seawater collected across the South Atlantic/Drak	CLE-CSV	HMDE	Natural pH	$34.1 < S < 34.8$	$c_{T,Cu} = 1.9$ to 2.6 nmol/L	1-2 $\mu\text{mol/L}$ SA + 10 mmol/L EPPS. Uniform (25-50 nmol/L) concentration of 1 relatively weak ligand.	[26]

	e Passage							
Cu	Seawater collected across the North Atlantic	CLE-CSV	HMDE	Natural pH	$34.5 < S < 38$	$c_{T,Cu} = 0.4$ to 3.1 nmol/L	2 or $2.5 \mu\text{mol/L}$ SA + HEPES/EPPS. Cu^{2+} found to range from 1.54 fmol/L to 1.07 pmol/L showing high complexation throughout the water column in the Atlantic. On-board analysis.	[25]
Zn	Western North Pacific	CLE-CSV	HMDE	8.2	$32.4 < S < 35.3$	$c_{T,Zn}$ from 0.03 to 2.11 nmol/L	$25 \mu\text{mol/L}$ APDC + Borate buffer. Some of the ligands attributed to bacteria and phytoplankton.	[37]
Fe	Eastern Tropical South Pacific oxygen minimum zone	CLE-CSV	HMDE	8.1		$c_{T,Fe}$ up to 1.5 nmol/L	$7.3 \mu\text{mol/L}$ TAC + 5 mmol/L EPPS. No significant difference of $\log K$ between anoxic and oxic waters	[33]
Fe	Antarctic, Sea ice, brine, snow and seawater	CLE-CSV	HMDE	8.3		$c_{T,Fe} = 0.3$ (brine) to 81.0 nmol/L (sea ice)	$5 \mu\text{mol/L}$ NN + borate buffer. $<99\%$ Fe complexed, possibly by polysaccharides.	[32]
Zn	GSH and root extracts of <i>Hordeum vulgare</i>	AGNES	HMDE	3.5 to 8	0.1 KNO_3	$[\text{Zn}^{2+}] = 20$ nmol/L to 1.5 mmol/L	Device using N_2/CO_2 saturated with water to control the evaporation and to fix the pH.	[134]
Fe	Mersey estuary + Liverpool Bay (UK)	CLE-CSV	HMDE	8.15	$18.8 < S < 30.9$	$c_{T,Fe} = 4.80$ to 84 nmol/L	$5, 10, 15, 25$ and $50 \mu\text{mol/L}$ SA + Borate buffer. Agreement between multiple and single analytical windows suggests the presence of only 1 strong class of ligand.	[51]
Fe	Seawater enriched with	CLE-CSV	HMDE	8.1	seawater	Varying	$10 \mu\text{mol/L}$ TAC + EPPS buffer. Look at the potential role of EPS in	[135]

	EPS						contributing as a major Fe ligand in marine waters.	
Zn	Waters from rivers Garonne, Gave de Cauterets and Gave de Pau in the Pyrenees	AGNES	SPE	7.25 to 8.16	natural ionic strength with an addition of 0.01 M KNO ₃	[Zn ²⁺]= 2.0 to 451 µg/L	Methodology for on-site measurement in freshwaters. Crossvalidation of on-site and laboratory results.	[109]
Pb	Polystyrene sulfonate and Iminodiacetate	AGNES	Bi film electrode (BiFE)	4 6	0.01 NaNO ₃	[Pb ²⁺]= 20.4 to 63.6 nmol/L [Pb ²⁺]= 0.084 to 0.188 µmol/L	Application of AGNES with mercury-free electrodes. Theoretical framework to apply AGNES when the activity is related to a surface concentration (instead of a volumic one).	[110]
Fe	Estuary, In-shore and off-shore stations in the China Sea	CLE-CSV	HMDE	8.1	31.7 < S < 34.1	c _{T,Fe} from 3 to 126 nmol/L	100 µmol/L DHN + POPSO and bromate. 11 < Log K' _{FeL} < 14 Higher surface concentrations of the ligands observed in the spring.	[30]
Cd	TiO ₂ nanoparticles	AGNES and SSCP	TMFE	6 to 8.5	0.01 NaNO ₃	[Cd ²⁺]= 0.01 to 1.0 µmol/L	AGNES and SSCP determined a homogeneous and labile adsorption complex of Cd ²⁺ to TiO ₂ nanoparticles.	[122]
Cu	Seawater	CSV	HMDE	8.2	S = 35	Saturation of HS with addition of Cu	No added ligand. New method for the determination of Cu-HS substances based on their specific adsorption on the Hg electrode.	[24]

Cu	Aqueous extract of <i>Eichhornia crassipes</i>	CLE-CSV	HMDE	8	Not given	$c_{T, Cu} = 144\text{--}480 \text{ nmol/L}$	SA ligand + borate buffer. Comparison of forward and reverse titrations.	[23]
Cu	UV seawater equilibrated with sediments	CLE-CSV	HMDE	8.1	Natural salinity. Coastal seawater	$c_{T, Cu}$ ca. 2 nmol/L	5 $\mu\text{mol/L}$ SA + 10 mmol/L Borate buffer. Cu complexing ligand released from sediments may account for 13% of the total ligand budget in coastal waters of the Tasman Sea.	[22]
Cd and Pb	Natural organic matter from two rivers in Brasil	AGNES and SSCP	TMFE on screen printed electrode	pH below 3 for total and 6.2 and 6.7 for the two rivers.	0.01 NaNO ₃	$[Cd^{2+}] = 3 \text{ to } 300 \text{ nmol/L}$	Development of a speciation methodology for total metal determination by SCP (acidification), free metal by AGNES and small complexes in the ultrafiltered solution (<1 kDa).	[90]
Cu	Malonic Iminodiacetic acids	AGNES	VGME	4 to 6	0.01 NaNO ₃	$[Cu^{2+}] = 15 \text{ to } 90 \text{ nmol/L}$ $[Cu^{2+}] = 26 \text{ to } 92.7 \text{ nmol/L}$	First use of Au-electrode in AGNES. Crossvalidation of AGNES with SSV.	[111]
Zn	Tamar river estuary	AGNES and CSV	HMDE	7.2 to 8.6	natural ionic strength	$[Zn^{2+}] = 4 \text{ to } 26 \text{ nmol/L}$	Values of AGNES crossvalidated with CLE-AdCSV. Application of AGNES with various salinities.	[38]
Cd Zn	Laurentian Fulvic Acid	AGNES	TMFE	6 to 8	NaNO ₃ 10 mM	$[Cd^{2+}] = 0.005 \text{ and } 5 \mu\text{mol/L}$ $[Zn^{2+}] = 0.005 \text{ and } 5 \mu\text{mol/L}$	AGNES was used to measure the free metal and obtain the NICA-Donnan parameters for Cd and Zn for Laurentian Fulvic Acid.	[120]

Cu	Estuarine waters	CLE-CSV	HMDE	8.2	Varying from freshwat er to seawater	$c_{T, Cu} = 2.2$ nmol/L ($S = 34.5$) to 27.5 nmol/L ($S = 4.8$)	2.5 μ mol/L SA + Borate-ammonium buffer. Use of reverse titration. Look at the size-fractionated alteration of Cu complexing capacity of peat-land humics with residence time and along S gradient	[21]
Cu	Estuarine waters	CLE-CSV	HMDE	7.8	Varying from freshwat er to seawater	$c_{T, Cu} = 8$ to 189 nmol/L	SA ligand (2 and 10 μ mol/L) + HEPES buffer. Shows that CLE-CSV results match VM prediction within 1 order of magnitude.	[20]
Cd Cu	Aldrich Humic Acid and MES	AGNES and SSCP	HMDE	6	10 mM or 100 mM KNO_3 or $Ca(NO_3)_2$	Not specified in the article	SSCP and AGNES used to determine the concentraation of free metal ion in the bulk.	[127]
Cd Pb Cu	Aldrich Humic Acid and MES	AGNES and SSCP	HMDE	6	10 mM or 100 mM KNO_3 or $Ca(NO_3)_2$	$[Cd^{2+}] = 3.2$ to 0.3 μ mol/L $[Pb^{2+}] = 0.77$ μ M to 65 pmol/L $[Cu^{2+}] = 1.2$ μ mol/L to 0.4 nmol/L	SSCP and AGNES used to determine the concentraation of free metal ion in the bulk.	[93]
Zn	Wine and synthetic solutions	AGNES	HMDE	3.4	natural ionic strength	$[Zn^{2+}] = 1.7$ μ mol/L and $[Zn^{2+}] = 6.5$ μ mol/L	Values of AGNES crossvalidated with DMT.	[112]
In	NTA	AGNES	HMDE	3.0	0.1	$[In^{3+}] = 9.84$	First application of AGNES with a trivalent	[105]

	oxalate			3.0 to 3.1	KNO ₃ 0.1 KNO ₃	μmol/L to 158 pmol/L [In ³⁺]= 87.9 μmol/L to 217 pmol/L	cation.	
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Table 2. Comparison of strong and weak characteristics of the selected electroanalytical techniques.

Issue	CLE-CSV	SSCP	AGNES
Pre-treatment	Buffer addition + Added ligand + possibly purging	Purging is necessary to avoid local pH increase.	Purging is necessary to avoid local pH increase [112;115;132]
Time of experimental determination	Each titration requires a minimum of 12 samples that must be prepared, equilibrated and analysed. Depending on those, experimental time can be significant. Recent recommendation includes the use of multiple analytical windows (i.e. multiple titrations) which will further this analysis time [49]. The use of fully automated apparatus would help increasing sample throughput.	A well-defined full SSCP wave needs roughly 15 individual SCP determinations. Due to the nature of the reoxidation step, the more adequate electrodes are thin-film electrodes and for those the reoxidation time is negligible when compared with the deposition time. Typical deposition times are of the order of 45-180s, so that an SSCP curve requires between 20 min and 1h. [75]	Depends on free concentration, on lability of complexes and on the size of the electrode. In the worst scenario, when complexes do not contribute at all, with HMDE, a rough rule is a deposition time (in seconds) 7 times the gain (Y) [101]. Zn from dissolving ZnO nanoparticles (in excess) at pH 7 could be measured every 5 minutes [124].
Interpretation of signal	Various methods (linear, non-linear, direct) for data fitting exist and it can be difficult to know which one to use. A recent inter-comparison of these methods has identified best procedures [49] and some software tools are recommended (e.g. [53] here). Strong experience is needed.	The potential shift in SCP follows a DeFord-Hume type equation providing the stability constants. The reoxidation time depends on kinetic and mass transport in solution. Comparison between the two signals provides experimental information on the lability of the complexes[79].	Very easy. The free concentration is just proportional to the analytical signal (e.g. current or charge) with a proportionality factor from a calibration
Limit of Detection	Low, due to accumulation at the surface of the sensor (no diffusion within the sensor during stripping).	Depends on the electrode geometry and hydrodynamic nature of the electrochemical cell. Best lower detection limit reported for	Depends on same characteristics as “Time of experimental determination” and on the time one can endure. pM range was achieved with Humic Acids

		SSCP is on the low nM level with 90s deposition time using a TMFE. For SCP the detection limit can reach pM levels[90].	[117].
On-field deployment	No. This is a laboratory technique that uses Hg drop electrode	Possible as long as the three electrode system uses a true reference electrode	Possible with SPE. [109]
Interferences from other metals or organic matter	DOC might interfere, possibly affecting the sensitivity at ligand saturation and, thus, affecting final results.	Possible signal superposition between Cd, Pb and In in natural media especially in presence of NOM.	Non-existing or avoidable with AGNES-SCP (except for In when Pb is at a similar level or very high interferent/analyte ratio) [104].
Electrode adsorption interference	High DOC might interfere with the sensitivity, thus affecting results.	Negligible effect whenever the charge for a full coverage is negligible with respect to the total deposited charge[73].	Non-existing, except if there is total blockage of the electrode surface. [103].
Impact of ionic strength	Must be kept constant. Measurements provide speciation information at specific ionic strength and pH.	Needs a calibration performed at the exact ionic strength of the determination.	Can be predicted, so there is portability of calibration across ionic strengths[102]
Elements to which can be applied	Current elements are Fe, Cu, Zn, Co, Ni, Pb but potentially applicable to many others.	Zn, Pb, Cd, Hg, Cu, Sn, In, Tl.	Existing results for: Zn, Pb, Cd, Cu, Sn, In[105], Sb. Future candidates: Tl, Ga.

Figure

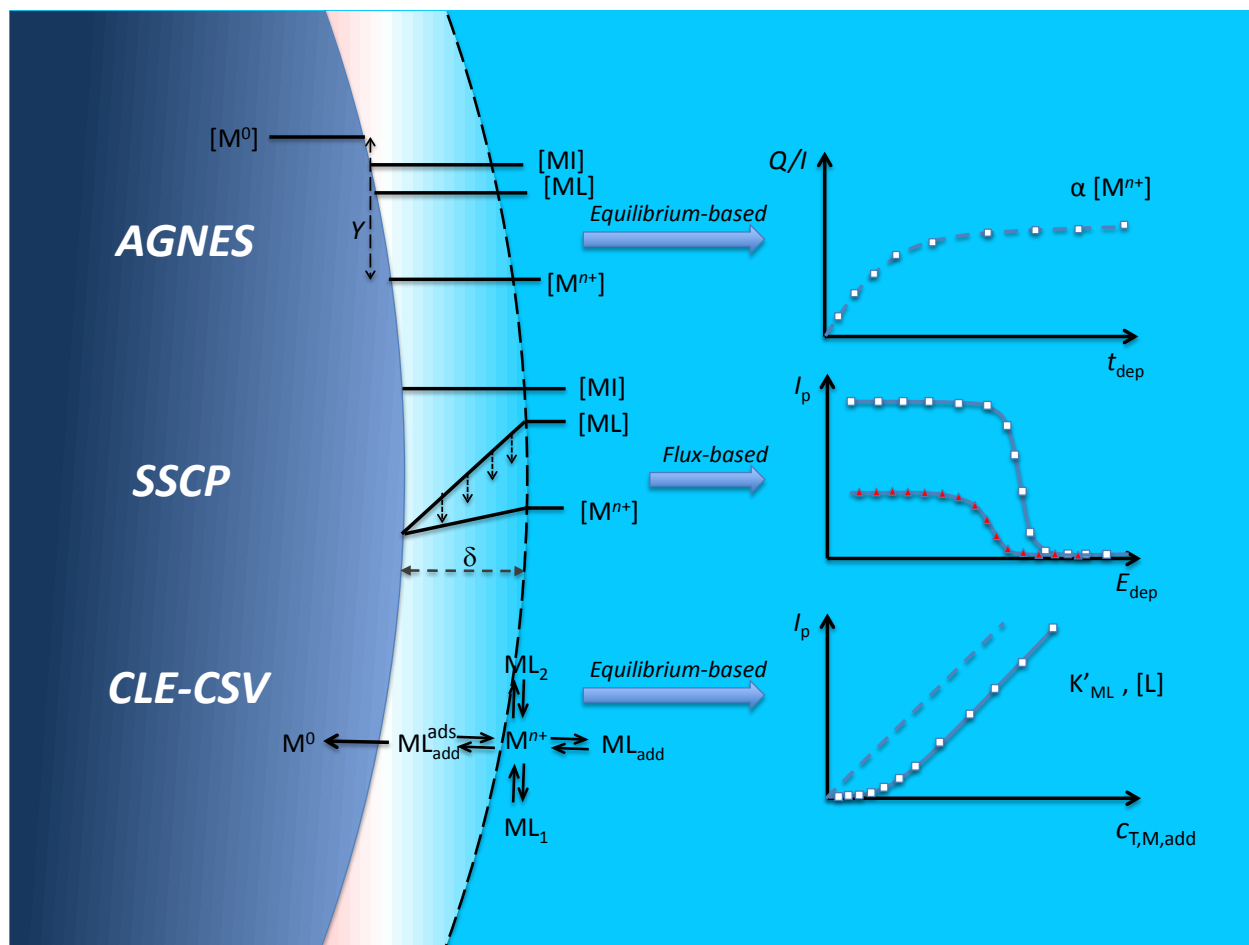


Fig 1 : Schematic outline of the selected electrochemical techniques of this review. A mercury electrode is represented on the left. Some relevant concentration profiles are depicted at the interphase with the solution, while typical outputs can be seen on the right.

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